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The Hows and Ys of Genome Integrity

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Much of the Y chromosome consists of large palindromic arrays harboring genes that are critical for spermatogenesis. In this issue, Lange et al. (2009) show that although gene conversion within these arrays maintains their integrity, it also permits rare unequal sister chromatid-exchange events within palindromes that create unstable dicentric chromosomes, resulting in infertility, sex reversal, and Turner syndrome.

To steal a phrase from an old Eagles song, "…every form of refuge has its price." Perhaps nowhere is this sentiment more true than in the case of the human Y chromosome. This small and primarily heterochromatic chromosome possesses a recombination-based mechanism for protecting the integrity of the genes it carries. In this issue of *Cell*, Lange et al. (2009) show that the mechanism that protects the Y chromosome also carries a cost—it allows the occurrence of rare sister chromatid exchanges that promote chromosomal instability.

The Y chromosome has two primary functions: to confer maleness during embryonic development via the expression of the *Sex-Determining Region Y* (*SRY*) gene and to carry a number of genes required in multiple copies for male fertility (Lahn et al., 2001). As critical as those functions may be, the Y chromosome is perpetually purported to be on the verge of extinction (Aitken and Marshall Graves, 2002). The problem lies in the fact that 95% of the Y chromosome does not recombine with its X chromosome homolog, thus allowing deleterious mutations to accumulate on the Y chromosome. Indeed, the male-specific region of the Y chromosome (*MSY*) was often described as a wasteland, only containing gene-poor heterochromatin composed of simple repeats that have little or no phenotypic effects when deleted (Lahn et al., 2001). This view of the Y chromosome changed with the discovery of seven ampliconic regions containing families of Y-specific genes of variable copy numbers. The discovery of these amplicons, which contain genes required for male fertility, suggested a mechanism for Y chromosome maintenance (Skaletsky et al., 2003). Within these ampliconic regions of the Y chromosome are eight massive palindromes composed of two large arms with >99% sequence identity that are separated by a spacer sequence. The sequence identity in these palindromes is maintained by frequent gene conversion between the repeated regions. Thus, although the Y chromosome may not be able to recombine with a homologous chromosome, intrachromosomal gene conversion allows functional copies of a given gene within the palindrome to "correct" a mutant copy. Although some gene conversion events could eventually greatly increase the number of mutant alleles within the palindrome, the resulting mutant Y chromosomes from these events would be "lost" as these males would be sterile and thus unable to pass on the chromosome (Skaletsky et al., 2003).

By examining Y chromosomes from male human patients who cannot make enough sperm, have Y chromosome structural anomalies, or have sex reversal (where the physical phenotype does not correspond to the sex chromosome genotype), Lange et al. now show that this mechanism of Y chromosome preservation may come at a cost. Indeed, the arrangement of genes in long palindromic arrays and the frequent occurrence of recombination events within the arrays pose the risk that the repair products of double-strand breaks could be resolved through the crossover pathway of homologous recombination rather than the noncrossover process of gene conversion. If the double-strand breaks are processed to produce crossovers, an exchange between similarly oriented repeats on sister chromatids can produce an isodicentric Y (idicY) chromosome—a "mirror-image" chromosome that contains two centromeres with an axis of symmetry through the center of the palindrome containing the break (Figure 1A). The inherent instability of idicY chromosomes could cause Y chromosome breakage and loss during chromosome segregation (Figure 1B). In a beautiful cytogenetic analysis of idicY chromosomes in human patients, Lange et al. demonstrate the occurrence of exactly such unequal sister exchanges, albeit at a very low frequency. They thus demonstrate that both crossover and noncrossover recombination pathways are active in the palindromic regions of the Y chromosome.

In total, Lange and colleagues identified 51 unrelated patients with idicY chromosomes whose structures are consistent with being formed as a result of homology-mediated crossover between the palindromes of sister chromatids. Of the eight palindromes in *MSY*, breakpoints leading to idicY formation were found in seven of the palindromes. After identifying these idicY chromosomes by utilizing unique Y chromosome genetic markers, the authors confirmed the mirror-image nature of these chromosomes by fluorescent in situ hybridization analysis of metaphase and interphase cells.

By definition, isodicentric chromosomes have two centromeres. How can a chromosome with two centromeres sur-

vive? If each of the centromeres remains active, the dicentric Y chromosome may be snapped apart during chromosome segregation (Figure 1B), leading to its damage or loss (McClintock, 1941). Alternatively, it is also possible for one centromere of a dicentric chromosome to become inactive, thus helping to ensure faithful segregation of the chromosome (Page and Shaffer, 1998). When examining chromosome spreads of cells from idicY patients, Lange et al. find that cells harboring idicY chromosomes with very short distances between centromeres (4.5–9.1 Mb) can be categorized into two types. Some cells contain idicY chromosomes with one active centromere and other cells contain idicY chromosomes with two active centromeres. Presumably, close proximity between centromeres may allow two active centromeres (as identified by the presence of the centromeric protein CENP-E) to behave as one. Interestingly, Lange et al. observe that when the centromeres are more than 12.4 Mb apart, the idicY chromosome consistently contains only one active centromere.

IdicY chromosomes harbor deletions for some genes on one chromosome arm and duplications for some genes on the other chromosome arm, with the extent of deletion or duplication dependent on the location of the original breakpoint. Those Y chromosomes with deletions in the short arm (idicYq) lack the *SRY* gene that is needed for sex determination. As expected, Lange and colleagues find that individuals harboring idicYq chromosomes are phenotypically female. Curiously, some of the patients with idicY chromosomes that contain two copies of *SRY* (idicYp or isoYp) have both a degenerate ovary and a testis. The authors show that for the majority of these intersex patients, their idicY chromosomes have larger distances between centromeres in comparison to idicYp patients who are phenotypically male. Lange et al. interpret the often intersexual phenotype of idicYp or isoYp patients as being due to mitotic instability of the idicYp chromosome. This instability can lead to Y chromosome loss and mosaicism, such that some cells in these intersex patients lose the idicY chromosome and thus have only 45 chromosomes (45,X cells).

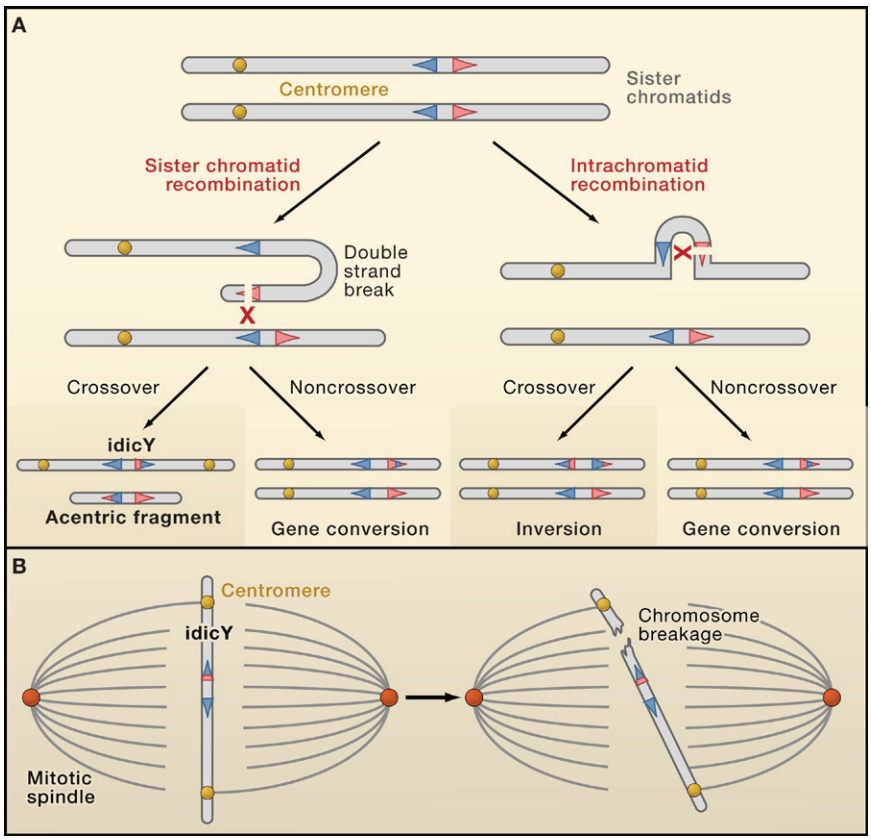

Figure 1. Resolution of Double-Strand Breaks in the Y Chromosome

(A) In the male-specific region of the Y chromosome (*MSY*), double-strand breaks can be repaired using either a sister chromatid or the corresponding arm of a palindrome within a chromatid. Recombination events can then be resolved via the crossover pathway (where regions flanking the break site are exchanged) or the noncrossover pathway. Regardless of the exchange partner, the noncrossover pathway produces gene conversion events. However, crossover between sister chromatids produces a dicentric chromosome and an acentric fragment, whereas crossover between palindromic repeats within a chromatid produces an inversion.

(B) If each centromere of a dicentric chromosome attaches to a different spindle pole, the chromosome can be pulled apart during chromosome segregation, leading to the loss of genetic material.

Of important clinical consequence is the instability and mitotic loss of the idicYp chromosome and the resulting formation of 45,X cells, which might result in individuals that exhibit symptoms of Turner syndrome, a phenotypically diverse condition typically caused by loss of all or part of a sex chromosome (Guedes et al., 2006). This would explain the observation that, unlike most trisomy-induced disorders where an error in maternal meiosis leads to the presence of an extra chromosome, the majority of Turner syndrome cases are due to the loss of a paternal chromosome (Jacobs et al., 1997). The findings of Lange et al. suggest that in some cases, the paternal origin of Turner syndrome may not lie in errors in spermatogenesis but rather may be due to the loss of an idicYp chromosome during early development of the zygote.

What does all of this mean for the fate of the Y chromosome? Although the lack of homologous recombination for 95% of the Y chromosome would seem to put it at an evolutionary disadvantage, the discovery of palindromic recombination in the *MSY* locus shows that the Y chromosome is intent on surviving longer than its predicted 10 million year expiration date (Aitken and Marshall Graves, 2002; Wilson and Makova, 2009). Despite the immense importance of the gene conversion events in the *MSY* locus, this recombination comes at a cost. Although crossover resolution of recombination events between palindromes on sister chromatids is rare (occurring in ~2% of

the patients with spermatogenic failure, Y chromosome anomalies, or sex reversal examined by Lange et al.), it is a clear drawback to the Y chromosome's mechanism of self-preservation. Indeed, every form of refuge really does have its price.

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The Rea1 Tadpole Loses Its Tail

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More than 170 assembly factors aid the construction and maturation of yeast ribosomes. After these factors' functions are completed, they must be released from preribosomes. In this issue, Ulbrich et al. (2009) describe a mechanochemical process through which the AAA ATPase Rea1 induces release of an assembly protein complex from preribosomes.

Ribosomes, the ubiquitous factories that produce proteins from mRNAs, are essential for growth, proliferation, and adaptation of cells. In eukaryotes, assembly of these complex ribonucleoprotein particles (RNPs) begins in the nucleolus with the association of a subset of ribosomal proteins (r-proteins) and *trans*-acting assembly factors with the nascent ribosomal RNA (rRNA) to form the 90S pre-rRNP, the single precursor to both the 40S and 60S mature subunits. The assembly factors are transient actors—they are released once their role is completed. But do they just know when to let go or are they actively removed from the maturing subunits? In this issue, Ulbrich et al. (2009) provide the most detailed study to date to answer this question. They discover a mechanochemical process for release of assembly factors and suggest that release is an integral part of ribosomal subunit maturation.

Beginning with the 90S precursor, the pre-rRNP undergoes a series of pre-rRNA processing and assembly steps while transiting from the nucleolus through the nucleoplasm to the cytoplasm to form mature 40S and 60S functional subunits (Henras et al., 2008). The two ribosomal subunits contain intricate structural cores of rRNA decorated on their surfaces with r-proteins. Studies of bacterial ribosome assembly in vitro revealed that ribosomal subunit assembly is cooperative and hierarchical. Ribosomal RNA and bound r-proteins undergo multiple structural rearrangements induced by binding of additional r-proteins to enable successive assembly steps (reviewed in Nomura, 1990). However, binding of r-proteins to rRNA is not sufficient to drive assembly forward.

Genetic and proteomic analysis in yeast has identified >170 proteins present in pre-rRNPs, but not mature ribosomes, that are required for ribosome assembly in vivo. These assembly factors, largely conserved from yeast to humans, include AAA ATPases, GTPases, RNA-dependent ATPases/helicases, kinases, nucleases, scaffolding proteins, and RNA-binding proteins. At least nine of these proteins, including GTPases and ATPases, release other factors, reduce the complexity of pre-rRNPs, and power the progression of subunit maturation (Figure 1; reviewed in Henras et al., 2008; Zemp and Kutay, 2007).

Although we now have a great deal of insight into what mature ribosomal subunits look like, understanding the mechanism of ribosome assembly in detail requires learning the precise functions of each assembly factor. Several key questions immediately come to mind: At which point in the assembly pathway does each factor associate with pre-rRNPs? Where is each factor located in preribosomes? When does each factor function? Upon which molecules does each factor act? When, how, and why do assembly factors exit from pre-rRNPs?

In their new study, Ulbrich and coworkers use an elegant combination of electron microscopy, site-directed mutagenesis, and assays of factor release from preribosomes to answer these questions about the AAA ATPase Rea1. These analyses enable them to work out how Rea1 operates in ribosome biogenesis.